

## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Amyotrophic lateral sclerosis and the innate immune system: Protocol for establishing a biobank and statistical analysis plan
<b>AUTHORS</b>	Kjældgaard, Anne-Lene; Pilely, Katrine; Olsen, Karsten Skovgaard; Lauritsen, Anne Øberg; Pedersen, Stephen Wørlich; Møller, Kirsten; Garred, Peter

### VERSION 1 - REVIEW

<b>REVIEWER</b>	JESSICA MANDRIOLI AZIENDA OSPEDALIERO UNIVERSITARIA DI MODENA, ITALY
<b>REVIEW RETURNED</b>	16-Mar-2020

<b>GENERAL COMMENTS</b>	<p>The authors here present a prospective, observational case-control study, where they will collect samples from ALS patients, neurologically healthy controls, and non-ALS neurological controls, with the aim of establishing a biobank and of carrying out 4 trials and possibly further studies.</p> <p>I have several concerns:</p> <ul style="list-style-type: none"><li>- The study is focused on establishment of a ALS biobank, but it seems to me more like a collection of samples to perform different studies. This is not a biobank, as biobanks should comply a set of requirements regarding governance, facilities, infrastructures, data sharing, sample sharing policies, record management policies, that are not given here. See for example "Recommendations for biobanks - IARC Publications"</li><li>- Protocol papers should report planned or ongoing studies. The dates of the study should be included in the manuscript, and I cannot find them, whereas it is stated that ethics and DPA approval have already been obtained. Moreover, the authors report Trial registration with an initial release: 06/28/2016 (four years ago)</li><li>- Clinical phenotyping is quite limited; spontaneous ALS should be changed with sporadic ALS. The authors does not give details on neuropsychological testing ("Furthermore, it is noted whether the ALS specialists observe any sign of cognitive impairment", this is not acceptable, how do they define any sign of cognitive impairment?)</li><li>- The authors state that they "aim to develop and validate a new, simple, early progression score based on the ALSFRS-R score", but they do not explain it anymore, whereas they explain the 4 biological trials aims .</li><li>- I don't understand why are they excluding some neurological diseases from neurological controls...</li><li>- I don't understand the sentence "However, some patients have symptoms of a slower progressing motor neuron disease and therefore get the diagnosis of primary motor neuron disease", and</li></ul>
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	<p>the discussion on inclusion criteria.</p> <p>In conclusion the paper is quite confusing and imprecise in some parts (clinical and phenotype data collection, biobank requirements, etc..)and lacks of some important data stating that the study is still going on and not finished.</p>
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<b>REVIEWER</b>	<p>Aisha Dickerson</p> <p>Johns Hopkins Bloomberg School of Public Health</p>
<b>REVIEW RETURNED</b>	03-Apr-2020

<b>GENERAL COMMENTS</b>	<p><b>Abstract:</b></p> <p>It is not clear what the authors mean by “neurological controls”. Are these people with other neurological disorders (i.e. Parkinson’s disease, multiple sclerosis, Alzheimer’s disease)?</p> <p>I also do not think it is necessary to state “All results will be published in peer-reviewed, medical journals and presented at scientific conferences”. Please remove this.</p> <p><b>Introduction:</b></p> <p>Page 3: Where did this median survival time come from? Is this global? Otherwise, a median should not consist of a range of numbers.</p> <p>Page 4, 1st line: “percent” is one word. The, authors should replace “familiar” with “familial”, and there should be a reference for this sentence.</p> <p><b>METHODS:</b></p> <p>Page 5, Design subsection: It appears that SPIRIT is an acronym for something. Please define what this acronym stands for and provide a reference for these guidelines.</p> <p>In Table 1, the authors should define the acronyms (NC, NHC) in the footnote or use the space provided to use the entire phrase that the acronym stands for.</p> <p>Page 5, Participants subsection: The authors should define what other neurological diseases they are referring to.</p> <p>Page 6, Exclusion criteria subsection: Why were patients with motor neuron disease exclude? Do the authors mean that these people were included in the ALS group? If not, please provide an explanation.</p> <p>Page 7, Settings subsection: The authors should not start each sentence on a separate line. As they are pieces of the same topic, they should be combined into one paragraph.</p> <p>Page 7, Data collection subsection: Similarly, the authors should not start each sentence on a separate line. As they are pieces of the same topic, they should be combined into one paragraph.</p> <p>Page 7-8 Biological samples section: Similarly, the authors should not start each sentence on a separate line. As they are pieces of the same topic, they should be combined into one paragraph. Please revise this throughout.</p> <p>Page 8, Muscle biopsies subsection: the authors should not use an abbreviation for department. Please use the complete word.</p> <p>Outcome measure section: By definition clinical trials must have an intervention of some kind. As this paper is currently written, it is too difficult to figure out what the authors plan to do with the 4 “clinical trials” being presented. Thus, outcome measures should not be written as a separate section. Each “Clinical trial” should be described fully in its own section with subsections for outcome, intervention, statistical analysis and sample size/power. Also, the other sections of the paper (abstract, introduction, aims) indicate that</p>
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	<p>this is an observational case-control study, so I do not understand where the clinical trials are coming from.</p> <p>Statistical Analysis section: The authors write in present tense as if some of these analyses are in process. If so, some of the preliminary results should be presented in this paper.</p> <p>The discussion section is far too short. This section should answer the following questions: What is the purpose of building this project and biobank? Are there other similar projects/biobanks, and will this differ from those in any way? How long do the authors intend to maintain this biobank? Where will it be housed? Who will be in charge of securing the samples? What is the composition of the research team? How can potential collaborators seek to access these samples or data from the samples? Who will review data use requests? What data might be generated from the samples (GWAS, immune titers, metal concentrations, etc)? How will data be disseminated?</p>
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<b>REVIEWER</b>	Gianni Sorarù Department of Neurology, University of Padova, Italy
<b>REVIEW RETURNED</b>	13-Apr-2020

<b>GENERAL COMMENTS</b>	<p>This manuscript reports about the research project aimed at ultimately establishing an ALS biobank in Denmark. In this context, the authors will try to explore, among others, the involvement of complement system in the pathogenesis of ALS. Complement measurements will be conducted in biological samples, including blood, CSF and muscle, prospectively collected from ALS patients and both "neurological" and healthy controls. There are methodological flaws that should be reviewed. As first, the authors have to clarify the definition of neurological controls as it is not clear whether any other neurological disease, including neurodegenerative or inflammatory disorders, will enter this arm. Second, while I have no concern about plasma and CSF analysis, muscle investigations appear hardly feasible because of the rarity of NMJs in muscle specimens, especially if in ONE tru-cut muscle biopsies. This point must be discussed. Last but not least, no data about genetics is going to be collected. I believe this aspect should be implemented in the light of the the very long term aim of this project.</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:.

Reviewer: 1

Reviewer Name: JESSICA MANDRIOLI

Institution and Country: AZIENDA OSPEDALIERO UNIVERSITARIA DI MODENA, ITALY

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The authors here present a prospective, observational case-control study, where they will collect samples from ALS patients, neurologically healthy controls, and non-ALS neurological controls, with the aim of establishing a biobank and of carrying out 4 trials and possibly further studies.

I have several concerns:

- The study is focused on establishment of a ALS biobank, but it seems to me more like a collection of samples to perform different studies. This is not a biobank, as biobanks should comply a set of requirements regarding governance, facilities, infrastructures, data sharing, sample sharing policies, record management policies, that are not given here. See for example “ Recommendations for biobanks - IARC Publications”

RESPONSE: We fully intend to establish a bio-bank steering committee and to fulfill all the formalities regarding the bio-bank and we will continue the clinical studies in accordance with all the formalities. We have applied more details throughout the manuscript in order to emphasize the bio-bank facilities enabled by this project.

Protocol papers should report planned or ongoing studies. The dates of the study should be included in the manuscript, and I cannot find them, whereas it is stated that ethics and DPA approval have already been obtained. Moreover, the authors report Trial registration with an initial release: 06/28/2016 (four years ago).

RESPONSE: We are aware of the fact that this project has been initiated four years ago and our original time line for this project has not been followed. This is due to the fact that the primary investigator (the first author of this article) has been unable to work for long periods of time as her husband has been seriously ill. The project is still on-going – however much delayed. We plan to start recruitment for the two last substudies this fall which we have also noted now in the manuscript.

Clinical phenotyping is quite limited; spontaneous ALS should be changed with sporadic ALS.

RESPONSE: We have corrected this.

The authors does not give details on neuropsychological testing (“Furthermore, it is noted whether the ALS specialists observe any sign of cognitive impairment”, this is not acceptable, how do they define any sign of cognitive impairment?)

RESPONSE: We acknowledge this limitation. We have elaborated on this in the trial limitation section.

The authors state that they “aim to develop and validate a new, simple, early progression score based on the ALSFRS-R score”, but they do not explain it anymore, whereas they explain the 4 biological trials aims.

RESPONSE: Thank you for this valuable note; we have removed the sentence as this study is a future project that has not yet been planned in details.

I don't understand why are they excluding some neurological diseases from neurological controls...

RESPONSE: We have now explained this in the discussion section.

,  
I don't understand the sentence “However, some patients have symptoms of a slower progressing motor neuron disease and therefore get the diagnosis of primary motor neuron disease”, and the discussion on inclusion criteria.

RESPONSE: We have rewritten this and there is a new paragraph on this subject in the section entitled ‘Trial limitations’.

In conclusion the paper is quite confusing and imprecise in some parts (clinical and phenotype data collection, biobank requirements, etc..)and lacks of some important data stating that the study is still going on and not finished.

RESPONSE: We have described the bio-bank settings in more detail in relevant sections throughout the manuscript.

Reviewer: 2

Reviewer Name: Aisha Dickerson

Institution and Country: Johns Hopkins Bloomberg School of Public Health

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

Abstract:

It is not clear what the authors mean by “neurological controls”. Are these people with other neurological disorders (i.e. Parkinson’s disease, multiple sclerosis, Alzheimer’s disease)?

RESPONSE: We have tried to describe the neurological controls in better details in the section entitled ‘Participants’.

I also do not think it is necessary to state “All results will be published in peer-reviewed, medical journals and presented at scientific conferences”. Please remove this.

RESPONSE: The editor requires that this statement remains in the manuscript according to the publishing format.

Introduction:

Page 3: Where did this median survival time come from? Is this global? Otherwise, a median should not consist of a range of numbers.

RESPONSE: We have now clarified this so it is clear that this median survival time is global.

Page 4, 1st line: “percent” is one word.

RESPONSE: This has been corrected.

The, authors should replace “familiar” with “familial”, and there should be a reference for this sentence.

RESPONSE: The error has been corrected and the reference is the same as the one after the next sentence. I have now referred to the reference in the first sentence too.

METHODS:

Page 5, Design subsection: It appears that SPIRIT is an acronym for something. Please define what this acronym stands for and provide a reference for these guidelines.

RESPONSE: We have now defined the acronym in the text and in the list of abbreviations and referred to the guidelines.

In Table 1, the authors should define the acronyms (NC, NHC) in the footnote or use the space provided to use the entire phrase that the acronym stands for.

RESPONSE: This has been changed.

Page 5, Participants subsection: The authors should define what other neurological diseases they are referring to.

This has now been elaborated so that it is more clearly described.

Page 6, Exclusion criteria subsection: Why were patients with motor neuron disease exclude? Do the authors mean that these people were included in the ALS group? If not, please provide an explanation.

RESPONSE: We have changed the headline so it is more clear that the MND is an exclusion criterium for the two control groups. We have also described the dilemma of only including probable and definite ALS considering the course of other motor neuron diseases than ALS – for example primary lateral sclerosis – in the discussion section.

Page 7, Settings subsection: The authors should not start each sentence on a separate line. As they are pieces of the same topic, they should be combined into one paragraph.

RESPONSE: This has been changed.

Page 7, Data collection subsection: Similarly, the authors should not start each sentence on a separate line. As they are pieces of the same topic, they should be combined into one paragraph.

RESPONSE: This had been changed.

Page 7-8 Biological samples section: Similarly, the authors should not start each sentence on a separate line. As they are pieces of the same topic, they should be combined into one paragraph. Please revise this throughout.

RESPONSE: We have now put the sentences into one paragraph.

Page 8, Muscle biopsies subsection: the authors should not use an abbreviation for department. Please use the complete word.

RESPONSE: This has now been changed as accordingly.

Outcome measure section: By definition clinical trials must have an intervention of some kind. As this paper is currently written, it is too difficult to figure out what the authors plan to do with the 4 “clinical trials” being presented. Thus, outcome measures should not be written as a separate section. Each “Clinical trial” should be described fully in its own section with subsections for outcome, intervention, statistical analysis and sample size/power.

RESPONSE: We have now changed the sections accordingly.

Also, the other sections of the paper (abstract, introduction, aims) indicate that this is an observational case-control study, so I do not understand where the clinical trails are coming from.

RESPONSE: We have changed the names of the four substudies from ‘Clinical trials 1-4’ to ‘Substudy 1-4’ as we realize that we used the wrong terminology.

Statistical Analysis section: The authors write in present tense as if some of these analyses are in process. If so, some of the preliminary results should be presented in this paper.

RESPONSE: We do not have preliminary results to publish in this manuscript and have changed it accordingly.

The discussion section is far too short. This section should answer the following questions: What is the purpose of building this project and biobank? Are there other similar projects/biobanks, and will this differ from those in any way? How long do the authors intend to maintain this biobank? Where will it be housed? Who will be in charge of securing the samples? What is the composition of the research team? How can potential collaborators seek to access these samples or data from the samples? Who will review data use requests? What data might be generated from the samples (GWAS, immune titers, metal concentrations, etc)? How will data be disseminated?

RESPONSE: We have elaborated on these subjects in the discussion section.

Reviewer: 3

Reviewer Name: Gianni Sorarù

Institution and Country: Department of Neurology, University of Padova, Italy

Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below

This manuscript reports about the research project aimed at ultimately establishing an ALS biobank in Denmark. In this context, the authors will try to explore, among others, the involvement of complement system in the pathogenesis of ALS. Complement measurements will be conducted in biological samples, including blood, CSF and muscle, prospectively collected from ALS patients and both "neurological" and healthy controls. There are methodological flaws that should be reviewed. As first, the authors have to clarify the definition of neurological controls as it is not clear whether any other neurological disease, including neurodegenerative or inflammatory disorders, will enter this arm.

RESPONSE: We have tried to clarify the section about the neurological controls. In addition, we have discussed the choice of control groups in the discussion section.

Second, while I have no concern about plasma and CSF analysis, muscle investigations appear hardly feasible because of the rarity of NMJs in muscle specimens, especially if in ONE tru-cut muscle biopsies. This point must be discussed.

RESPONSE: We agree that one tru-cut muscle biopsy may obtain too little material for a study of neuromuscular junctions and we have addressed this in the discussion section.

Last but not least, no data about genetics is going to be collected. I believe this aspect should be implemented in the light of the very long term aim of this project.

RESPONSE: Thank for this valuable note. We have now elaborated on the genetics and this point in the discussion section.

## VERSION 2 – REVIEW

<b>REVIEWER</b>	Aisha Dickerson Johns Hopkins Bloomberg School of Public Health
<b>REVIEW RETURNED</b>	07-May-2020

<b>GENERAL COMMENTS</b>	All of my previous comments were sufficiently addressed. The manuscript has greatly improved. Thank you.
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<b>REVIEWER</b>	Gianni Sorarù Department of Neurosciences, University of Padova, ItalyNone declared
<b>REVIEW RETURNED</b>	08-May-2020

<b>GENERAL COMMENTS</b>	Manuscripts has been adequately revised
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